Casein haplotype variability in Apulian goat breeds

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Abstract. Due to the tight linkage among the casein genes, the study of the haplotype variability is a necessary approach in order to identify important effects which could be exploited for the genetic improvement of goat species, showing considerable casein genetic variation. The aim of this paper was to analyse the casein haplotype distribution, with particular attention to the linkage phase between α_{s1}-casein (*CSN1S1*) and β-casein (*CSN2*). The two first loci of the casein cluster, which are only 12 kb apart and are convergently transcribed. Apulian goats from Garganica, Jonica, and Maltese breeds were considered. DNA typing showed that the *CSN2*C* allele is mainly associated to *CSN1S1*A* and *CSN1S1*F* allele. Most probably, the differentiation between *CSN2*C* and *CSN2*A* occurred before the numerous mutations affecting *CSN1S1* locus.

Riassunto. Variabilità degli aplotipi caseinici in razze caprine pugliesi. A causa della stretta associazione tra i geni che codificano per le caseine, lo studio delle variabilità aplotipica è un approccio necessario per identificare importanti effetti che potrebbero essere usati per il miglioramento genetico della specie caprina, nella quale il polimorfismo caseinico è notevolmente elevato. Scopo del presente lavoro è analizzare la distribuzione delle combinazioni aplotipiche caseiniche, con particolare riguardo alla fase dell'associazione tra α_{s1}-caseina (*CSN1S1*) e β-caseina (*CSN2*). Si tratta infatti dei primi due loci del *cluster* caseinico, che distano solo 12 kb e sono convergentemente trascritti. Il lavoro ha riguardato le razze caprine Garganica, Jonica e Maltese di allevamenti pugliesi. Le tipizzazioni, condotte a livello di DNA, hanno evidenziato come l'allele *CSN2*C* sia prevalentemente associato alle varianti *CSN1S1*A* e *CSN1S1*F*, mentre *CSN2*A* è solitamente in associazione con *CSN1S1*B*. L'allele nullo *CSN2*0* è in generale associato alla variante *CSN1S1*A*. Molto probabilmente, il differenziamento tra *CSN2*C* e *CSN2*A* è antecedente alle numerose mutazioni che hanno interessato il locus *CSN1S1*.

Introduction. Goat casein genes are highly polymorphic (Chessa et al., 2003). Moreover, they are organized as a cluster (figure 1) including in the order α_{s1}-casein (*CSN1S1*), β-casein (*CSN2*), α_{s2}-casein (*CSN1S2*), and κ-casein (*CSN3*) (Ferretti et al., 1990; Threadgill and Womack, 1990). The entire casein gene cluster region spans about 250 kb on chromosome 6 (Hayes et al., 1993; Popescu et al., 1996). Furthermore, *CSN1S1* and *CSN2* are only 12 kb apart and convergently transcribed (Leroux and Martin, 1996).

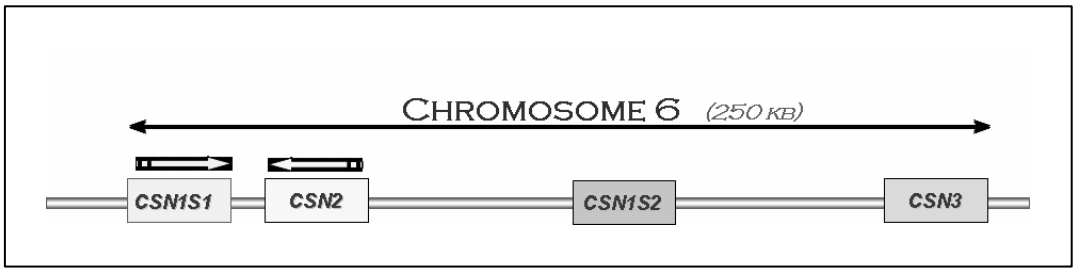

Figure 1: Goat casein cluster on chromosome 6.

Due to the tight linkage among the casein genes, the study of the haplotype variability is a necessary approach to identify important effects which could be exploited for the genetic improvement of the goat species. The aim of this paper was to analyse the casein haplotype distribution in Apulian goats from Garganica, Jonica, and Maltese breeds. The casein haplotype variability had been already considered in these breeds without taking into account *CSN2* locus (Sacchi et al., 2005), because a test for discriminating the two main alleles, *CSN2*A* and *CSN2*C*, was not yet available. Figure 2 represents the casein haplotype frequencies from Sacchi et al. (2005) in the three breeds. Chessa et al. (2005) provided the molecular test and demonstrated the predominance of *CSN2*C* in Italian breeds.

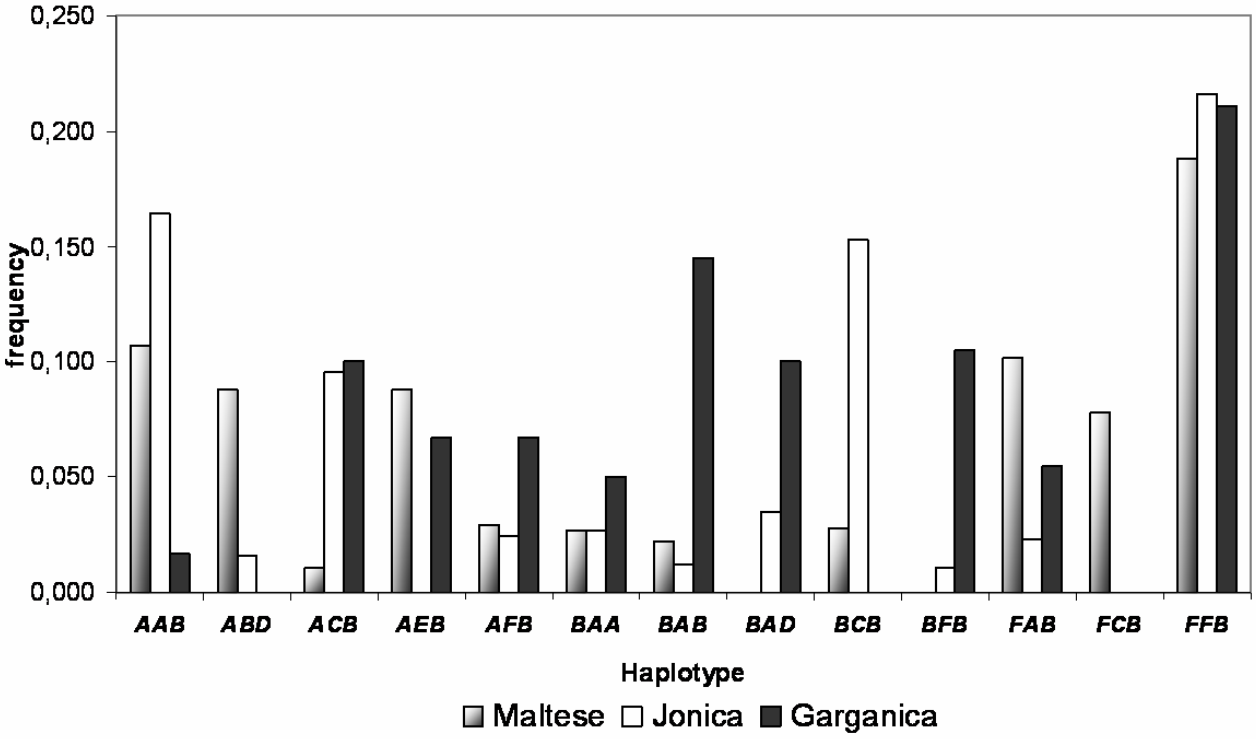

Figure 2: Haplotype frequencies at *CSN1S1-CSN1S2-CSN3* (from Sacchi et al., 2005). *CSN2* was not considered. The *CSN3* nomenclature proposed by Prinzenberg et al. (2005) is used here.

Material and Methods. In the present work, the PCR-SSCP test for *CSN2* (Chessa et al., 2005) was applied to a total of 102 goats from the three breeds whose DNA was still available from the former survey (Sacchi et al., 2005). Casein haplotype variability was then analysed. Particular attention was given to the linkage phase between *CSN1S1* and *CSN2*, due to their closeness in the casein cluster. The haplotype frequencies estimated taking into account linkage between the two loci, and the expected frequencies under the hypothesis of independence, were evaluated by EH program (Xie and Ott, 1993).

Results and Discussion. The DNA typing showed that the *CSN2*C* allele is mainly associated to *CSN1S1*A* and *CSN1S1*F* allele, while *CSN2*A* is usually in linkage phase with *CSN1S1*B*, and *CSN2*0* with *CSN1S1*A* (table 1). Possibly, the differentiation between *CSN2*C* and *CSN2*A* occurred before the numerous mutations affecting *CSN1S1* locus, as indicated in the evolutive model proposed in figure 3, which is based on the most common *CSN1S1-CSN2* haplotypes in the three breeds as well as on the highest discrepancies found between estimated and expected frequencies. An intriguing question is if the *CSN2*A* → *CSN2*C* differentiation in the casein cluster might have a causative role in the evolution of the other casein loci.

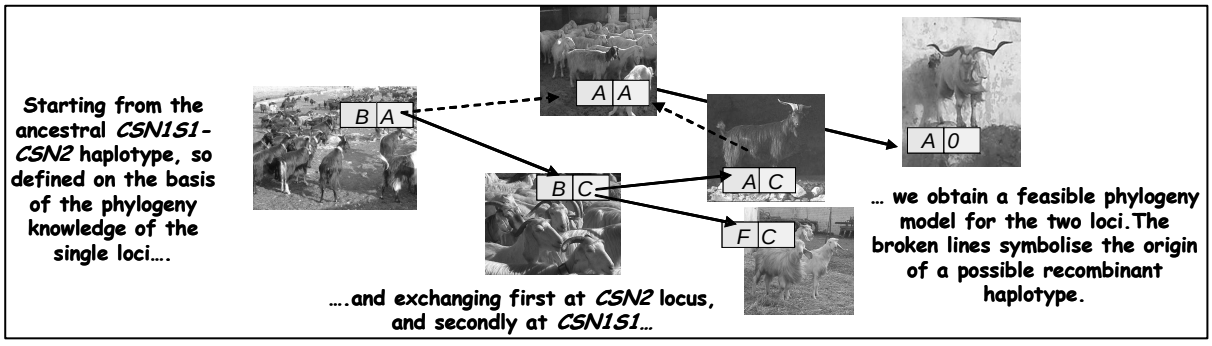

Figure 3: Evolutive pathway proposed in the text for *CSN1S1 – CSN2* haplotype.

CSN1S1	*CSN2*	Maltese (n = 54)	Jonica (n = 27)	Garganica (n = 21)	Overall (n = 102)
A	*A*	0.048	0.000	0.000	0.033
		0.050	*0.053*	*0.054*	*0.054*
A	*C*	0.323	0.446	0.238	0.314
		0.343	*0.374*	*0.218*	*0.325*
A	*0*	0.044	0.035	0.048	0.060
		0.023	*0.053*	*0.014*	*0.028*
B	*A*	0.072	0.084	0.124	0.089
		0.013	*0.023*	*0.063*	*0.024*
B	*C*	0.039	0.120	0.209	0.092
		0.092	*0.158*	*0.254*	*0.145*
B	*0*	0.000	0.000	0.000	0.000
		0.006	*0.023*	*0.016*	*0.012*
F	*A*	0.000	0.027	0.066	0.010
		0.057	*0.035*	*0.072*	*0.054*
F	*C*	0.461	0.212	0.315	0.393
		0.389	*0.245*	*0.290*	*0.329*
F	*0*	0.011	0.076	0.000	0.008
		0.026	*0.035*	*0.018*	*0.028*

Table 1: Haplotype frequencies at *CSN1S1*-*CSN2* estimated taking into account linkage between loci. In *italics*: haplotype frequencies under the hypothesis of independence.

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